

## SEM studies on seed morphology of *Indigofera* L. (Fabaceae) and its Taxonomic Utility

G. V. S. Murthy and M. Sanjappa\*

Botanical Survey of India, Southern Circle, TNAU Campus  
Lawley Road P.O., Coimbatore - 641 003, Tamil Nadu, India

\* Botanical Survey of India, P-8, Brabourne Road  
Kolkata - 700 001, West Bengal, India

---

### Abstract

Seed morphology of 51 species of *Indigofera* from India has been studied under SEM. The seeds display considerable diversity in shape, seed coat surface, size and numbers per pod in the genus. Based on the shape five major seed types viz., boat-shaped, bilateral, discoid, spherical and cylindrical have been recognised. The cylindrical type can be divided into two broad categories as pitted type and smooth type. The pitted type is further grouped into pitted-verrucate and pitted-smooth. The SEM study has revealed the remarkable topographic diversity of seed coat in different species. Seed surface ornamentation at higher magnification is variable, and is either smooth, pitted, stellate, reticulate, verrucate, granulate or perforate within the genus. On the whole, the surface pattern is found to be characteristic of each species. The present study not only provides some useful characters of seed for infrageneric classification but also for delimiting certain species of *Indigofera*. The occurrence of different shape types and seed coat patterns does not fully correlate with the taxonomical divisions, and indicates parallel evolution in *Indigofera*.

### INTRODUCTION

The genus *Indigofera* L. is the third largest genus in Leguminosae (Fabaceae), with c. 700 species distributed widely in tropical and subtropical regions of the world. A large number of species c. 550 occur in Africa (Schrire, 1995). India has about 60 species and 10 varieties, of which 14 species are endemic to India (Sanjappa, 1995). Majority of the species of *Indigofera* are perennial shrubs or herbs, rarely small trees or treelets viz., *I. heterantha* Wall. ex Brandis, *I. stachyoides* Lindl. and *I. zollingeriana* Miq.

*Indigofera* characterised by medifixed biramous hairs and apiculate anthers is morphologically diverse with regard to leaf, inflorescence, flower, fruit and number of seeds per fruit. Using these characters several authors proposed infrageneric classification of *Indigofera* (Willdenow, 1802; De Candolle, 1825; Wight & Arnott, 1834; Meyer, 1836; Harvey, 1862;

## G.V.S. Murthy and M. Sanjappa

Bentham, 1865; Baker, 1871, 1876; Taubert, 1894; Rydberg, 1923; Baker f. 1926; Gillett, 1958; Schrire, 1995). Wight and Arnott (1834) divided Indian species of the genus into 9 sections based on flower and fruit characters. While Baker (1876) classified the 33 species from India into 4 subgenera viz., *Acanthonotus* Benth., *Sphaeridiophora* Desv., *Euindigofera* Benth. and *Amecarpus* Benth., on the basis of pod characters and number of ovules. The subgenus *Euindigofera* was further divided into 6 'groups' viz., *Simplicifoliae*, *Sessiliflorae*, *Digitatae*, *Dissitiflorae*, *Tinctoriae* and *Paniculatae* generally based on leaf and inflorescence characters. The last group *Tinctoriae* was subdivided into 3 unrecognised subgroups based on leaflet and flower size characters. Gillett (1958) in his monograph on African *Indigofera* merged *Indigastrum* Jaub. & Spach and *Microcharis* Benth. with *Indigofera* and recognised 5 subgenera: *Acanthonotus* (Benth.) Benth., *Amecarpus* Benth. ex Harvey, *Indigofera*, *Indigastrum* (Jaub. & Spach) Gillett and *Microcharis* (Benth.) Gillett, on the basis of presence or absence of an indumentum on the petals, stamen morphology and the shape of the pod. He subdivided subgenus *Amecarpus* into two sections (*Amecarpus* and *Demissae*) and subgenus *Indigofera* into 3 sections (*Indigofera*, *Latestipulatae* and *Paniculatae*). The section *Indigofera* was further divided into 17 subsections. Gillett (1958) considered subsection *Tinctoriae* to have a central position in the divergence of the genus. This section is pantropical with its greatest development in the Sino-Indian region (Craib, 1913; Ali, 1977). De Kort and Thijssse (1984) and Sanjappa (1995) have refrained from any formal subdivision of *Indigofera* of Southeast Asia and India respectively. Schrire (1992) resurrected the genera *Microcharis* Benth. and *Indigastrum* Jaub. & Spach, based on cladistic analysis of the tribe *Indigoferae* in Africa and Madagascar. Schrire (1995) recognised 26 sections, 16 subsections and 15 groups (without assigning any rank) under the genus based on cladistic analysis of 57 characters. The delimitation of these subgenera and the concepts used to establish them have varied from author to author but it is apparent that they are taxonomically distinguishable.

The chromosome studies have shown that diploids ( $2n = 16$ ), tetraploids ( $2n = 32$ ), hexaploids ( $2n = 48$ ) and aneuploids ( $2n = 14$ ) occur in *Indigofera* (Darlington & Wylie, 1945; Frahm-Leliveld, 1960, 1962 & 1966; Bir & Sidhu, 1967; Singh & Roy, 1970; Bhatt & Sanjappa, 1975; Sanjappa & Bhatt, 1976, 1977; Sanjappa, 1977). Frahm-Leliveld (1962) found a general agreement between the number and size of chromosomes with the infrageneric classification of the genus by Gillett (1958). Large chromosomes with  $2n = 16$  in number occur near the centre of Gillett's scheme, while small chromosomes with  $2n = 14$  (probably due to aneuploidy) are found at the periphery, and generally in more herbaceous species. Both large and medium sized chromosomes are reported in members of the section *Tinctoriae*.

Ferguson and Strachan (1982) have found a columellar infratectum and a coarsely rugulate tectum in the pollen of robust shrubs or treelets, while a granular infratectum and a more finely perforate tectum in the pollen of herbaceous taxa.

The pods in *Indigofera* are dehiscent or indehiscent. They may be erect or spreading or delfexed and these positions are used as discriminatory characters. The pods are linear to globose, straight or arcuate, rounded to quadrangular in cross section. They are glabrous to velvety,

SEM studies on seed morphology of *Indigofera*

sometimes with disc-shaped glands (*I. barberi*, *I. glandulosa*, *I. karuppiana*, *I. pedicelluta*, *I. prostrata*, *I. santapau*, *I. thoathathrii*, *I. trifoliata* and *I. nummularifolia*) or with stalked glands (*I. argentea* and *I. colutea*). The endocarp is septate between the seeds, sometimes spotted with swollen red/black coloured cells rich in tannin and they can form pits on seed coat.

Corner (1951) noted that the Leguminosae have a great diversity of seed types, which may be a source of taxonomic and evolutionary data. External morphological features shared by legume seeds at maturity are testa and hilum. Embellishments on these parts may be useful in identification of legume taxa. The seed morphology perhaps may be as important as flower or fruit morphology in taxonomy if equal attention is given to it. The usefulness of seed surface as a diagnostic character depends on the level of variation present both within and between taxa.

The comprehensive bibliography of Brisson and Pterson (1977) on SEM studies of seeds and other works (Clarke & Jernstedt, 1979; Crow, 1979) indicate the value of the SEM for the study of small seeds and fruits. The aim of this paper is to examine the seed morphological characters of the genus *Indigofera* and their taxonomic utility.

MATERIALS AND METHODS

Seeds of 51 species of *Indigofera* were obtained from herbarium specimens at Central National Herbarium (CAL). Mature, dry seeds were rinsed with ethyl alcohol and mounted on aluminium stubs with double side tape and coated with gold in a Polaron E-5000 Sputter Coating Unit. Samples were then examined using Philips SEM 525 at the Central National Herbarium, Howrah. The whole surface of each seed was examined under the SEM and photographs were taken at two different magnifications : lower magnification to show whole seed and higher magnification to show fine surface details. The species studied here are those recognized by Sanjappa (1995); and their names in alphabetical sequence, the details of the specimens used for SEM and the observations are given in Table 1.

Table 1. Seed characters in Indian species of *Indigofera*

Species	No. of seeds per pod	Seed shape	Seed surface: low/high magnification	Average seed size in mm	Materials examined
1	2	3	4	5	6
<i>I. angulosa</i> Edgew.	1 - 4	B	smooth, reticulate-papillose	3 x 1.5	Sanjappa 9257, Mt. Abu, Rajasthan
<i>I. argentea</i> Burm. f.	4 - 6 - 8	BL	perforate with faint reticulation	1 - 1.5	Wadhwa 5167, Rajasthan

## G.V.S. Murthy and M. Sanjappa

1	2	3	4	5	6
<i>I. arrecta</i> Hochst. ex A. Rich.	4 - 8	C, et, er	smooth, matted type	1.5 - 1	Rao 95440, Karnataka
<i>I. aspalathoides</i> Vahl ex DC.	3 - 8	C, et	smooth, reticulate	1 x 1	Sastry 7797, Karnataka
<i>I. astragalina</i> DC.	3 - 6	C, et	pitted-verrucate, granulate	2.5 x 1.5	R.S. Rao 92602, Maharashtra
<i>I. atropurpurea</i> Buch.-Ham. ex Hornem.	6 - 10	C, er	smooth, rugulose	2.5 x 2.5	Lace 5517, Nagaland
<i>I. barberi</i> Gamble	2 - 4	B	rugose, rugose	1 - 1.5 x 1	J.L. Ellis 32470, Kurnool, Andhra Pradesh
<i>I. bracteata</i> Grah. ex Baker	7 - 10	C, et	smooth, reticulate	2 x 1.5	C.B. Clarke 15310, Khasia, Meghalaya
<i>I. cassioides</i> Rottler ex DC.	7 - 12	C, er	smooth, reticulate	2 x 1	Sanjappa 9510, Maharashtra
<i>I. coerulea</i> Roxb.	3 - 4	C, et	smooth, reticulate- granulate	2 x 1.5	W.A. Talbot 1700, Dharwar, Karnataka
<i>I. colutea</i> (Burm. f.) Merr.	5 - 12	C, et	pitted-verrucate, reticulate	1 - 1.2 x 1	Gamble 17768, Tamil Nadu
<i>I. constricta</i> (Thwaites) Trimen	6 - 8	B	smooth, striato- reticulate	2 x 1	V.S. Rama- chandran 61979, Kerala
<i>I. cordifolia</i> Heyne ex Roth	1 - 2 - 3	C, et, er	pitted-verrucate, psilate	1 x 1	Tiwari 774, Rajasthan
<i>I. dalzellii</i> T. Cooke	4 - 5 (7)	C, er	smooth, stellate	1 - 1.5 x 1	Sanjappa 9512, Maharashtra
<i>I. dosua</i> Buch.- Ham. ex D. Don	6 - 10	C, er	smooth, striate	1.5 - 2 x 1.5 - 2	V. Narayana- swami 2796, West Bengal

SEM studies on seed morphology of *Indigofera*

1	2	3	4	5	6
<i>I. exilis</i> Grierson & Long	6 - 10	C, er	smooth, reticulate	2 x 1.2	<i>G.H. Cave</i> 6857, Sikkim
<i>I. galegoides</i> DC.	15 - 18	C, et	smooth, reticulate	2 x 1.2	<i>C.E.C. Fischer</i> 3519, Kerala
<i>I. glabra</i> L.	10 - 12	C, et	pitted-granulate, reticulate	2 - 2.5 x 1 - 1.5	<i>Vijay Kumar</i> 705, Karnataka
<i>I. glandulosa</i> Wendl.	1 - 2	S	smooth, coarsely reticulate	2 x 2	<i>A.R.K. Sastry</i> 71369, Madhya Pradesh
<i>I. hebepetala</i> Benth. ex Baker	8 - 10	C, er	smooth, reticulate	2 x 1	<i>Gamble</i> 20799, Himachal Pradesh
<i>I. heterantha</i> Wall. ex Brandis	6 - 12	C, et	smooth, stellate- reticulate	1 - 2 x 8 - 1.2	<i>Ellis</i> 9584, Pehalgam, Jammu & Kashmir
<i>I. himalayensis</i> Ali	10	BL	smooth, reticulate	4 x 2 - 3	<i>Inaquat</i> 19314, Kagai Valley, Jammu & Kashmir
<i>I. hirsuta</i> L.	5 - 7	C, et	pitted-verrucate rugose	1.2 x 1.2	<i>M. Rama Rao</i> 2177, Quilon, Kerala
<i>I. hochstetteri</i> Baker	5 - 9	D	smooth, verrucate- reticulate	1.5 x 1.5	<i>A.N. Singh</i> 3105, Rajasthan
<i>I. karnatakana</i> Sanjappa	5 - 10	C, et	pitted, psilate	2 x 1	<i>B.K.V. Kumar</i> 1286, Belgaum, Karnataka
<i>I. karuppiana</i> Pallithanam	6 - 10	C, et	smooth, rugulose	1.5 x 1	<i>E. Vajravelu</i> , 43614, Tamil Nadu
<i>I. lacei</i> Craib	6 - 10	C, er	smooth, rugulose	1.5 - 2 x 1.5	<i>Mokim</i> 1162, Myanmar

## G.V.S. Murthy and M. Sanjappa

1	2	3	4	5	6
<i>I. linifolia</i> (L. f.) Retz.	1	S	verrucate, oblong- hexagonal areas	1 x 1	Sanjappa 9516, Maharashtra
<i>I. linnaei</i> Ali	2 - 3	C, et, er	smooth, reticulate	1 x 1	V.J. Nair 57132, Tamil Nadu
<i>I. longiracemosa</i> Bovin ex Baill.	3 - 6	C, er	pitted-verrucate striate	1.5 - 2 x 1 - 1.5	Lawson 304, Nagercoil, Tamil Nadu
<i>I. mysorensis</i> Rottler ex DC.	3 - 4	BL	smooth, psilate	2 x 2	Sanjappa 728, Bangalore, Karnataka
<i>I. nigrescens</i> Kurz ex King & Prain	6 - 9	C, er	smooth, rugulose	1.5 x 2	Joseph 48603, Arunachal Pradesh
<i>I. nummulariifolia</i> (L.) Livera ex Alston	1	B	verrucate, stellate- verrucate	1.5 - 1.8 x 0.5 - 1	Cleghorn s.n., Tamil Nadu
<i>I. oblongifolia</i> Forsskal	6 - 8	C, er	smooth, reticulate- papillose	2 x 1	Tiwari 993, Rajasthan
<i>I. parviflora</i> Heyne ex Wight & Arn.	8 - 14	C, er	smooth, coarsely reticulate	2 x 1.5	K. Hemadri 104327, BSI, Maharashtra
<i>I. pedicellata</i> Wight & Arn.	5 - 6	C, et, er	pitted-verrucate, striate	1.5 - 1	C.A. Barber 7533, Kodaikanal, Tamil Nadu
<i>I. prostrata</i> Willd.	5 - 8	BL	smooth, coarsely reticulate	1 - 1.5 x 0.8	V. Kumar 1287, Karnataka
<i>I. pseudoreticulata</i> Grierson & Long	6 - 8	C	smooth, stellate	1.8 x 1	J. Lal & Sanjappa 2181, Tawang, Arunachal Pradesh

SEM studies on seed morphology of *Indigofera*

1	2	3	4	5	6
<i>I. sesquipedalis</i> C.B. Clarke ex Sanjappa	5 - 8	C, et, er	smooth, reticulate	2 - 2.5 x 1.5	C.B. Clarke 18598, Meghalaya
<i>I. sessiliflora</i> DC.	2 - 6	S	smooth, smooth	1 x 1	B.V. Shetty 2327, Rajasthan
* <i>I. spicata</i> Forsskal	8 - 10	C	coarsely reticulate	2 - 2.5 x 1 - 1.5	C.B. Clarke 11242 B, Meghalaya
<i>I. stachyodes</i> Lindley	10	C, er	smooth, rugulose	2 x 1	Prain's collector 383, Sikkim
<i>I. suffruticosa</i> Mill.	4 - 5	C, et	pitted-smooth reticulate	2 x 1	King's collector CAL Ac. 111044, S. Andaman
<i>I. tinctoria</i> L.	6 - 12	C, er	smooth, psilate	2 x 1	Gollen 2, West Bengal
<i>I. tirunelvelica</i> Sanjappa	1 - 2	B	smooth, stellate- verrucate	1 x 1	Hooper & Ramaswamy 39230, Tirunelveli, Tamil Nadu
<i>I. trifoliata</i> L.	5 - 7	C, er	smooth, fossulate	1 - 2 x 1 - 1.5	U.R. Kamble s.n., Maharashtra
<i>I. trita</i> L. f.	6 - 10	C, er	smooth, reticulate	2 x 1.5	Chandrabose 53402, Tamil Nadu
<i>I. uniflora</i> Buch.- Ham. ex Roxb.	3 - 7	C, et	smooth, concentric rings	1x 1	Saldanha & Sreenath 4955, Karnataka
<i>I. vicioides</i> Jaub. & Spach	5 - 7	C, er, et	pitted-verrucate psilate	1 x 1	Hooper & Ramaswamy 38388, Tirunelveli, Tamil Nadu

## G.V.S. Murthy and M. Sanjappa

1	2	3	4	5	6
<i>I. wightii</i> Graham ex Wight & Arn.	8 - 12	BL	smooth, perforate fine reticulate	1 - 1.5 x 1	N.C. Nair 61005, Tamil Nadu
<i>I. zollingeriana</i> Miq.	16	D	smooth, rugose	2 x 2	Sikdar 175, West Bengal

B = boat shape; BL = bilateral; C = cylindrical; D = discoid; S = spherical; er = ends round; et = ends truncate.

\* Asian elements are considered as *I. hendecaphylla* Jacq. by Du Puy, Labat and Schrire (Kew Bull. 48: 724-727, 1993.)

## RESULTS AND DISCUSSION

In this work, seed morphology of 51 species of *Indigofera* are investigated taking into consideration the size, shape and surface texture using SEM. It has been found that SEM provides the exact nature of variations in the micromorphology (Table 1). The SEM analysis of whole seed and seed coat surface have revealed many taxonomically useful characters that help in distinguishing species of *Indigofera*. The data have further helped in grouping distinct seed morphotypes in *Indigofera*.

## Seed shape

On the basis of shape, the seeds can be placed broadly into 5 groups: (1) Spherical type, (2) Boat-shaped type, (3) Discoid type, (4) Bilateral type, and (5) Cylindrical type. Among the 51 species studied, the seeds are spherical in 3 species viz., *I. glandulosa* (Fig. 7, A), *I. linifolia* (Fig. 10, A) and *I. sessiliflora* (Fig. 14, A); boat-shaped in 5 species: *I. angulosa* (Fig. 1, A), *I. barberi* (Fig. 3, A), *I. constricta* (Fig. 4, E), *I. nummulariifolia* (Fig. 11, E) and *I. tirunelvelica* (Fig. 15, E); discoid in 2 species: *I. hochstetteri* (Fig. 8, E) and *I. zollingeriana* (Fig. 17, E); bilateral in 5 species: *I. argentea* (Fig. 1, C), *I. himalayensis* (Fig. 8, A), *I. mysorensis* (Fig. 11, A), *I. prostrata* (Fig. 13, A) and *I. wightii* (Fig. 17, C); and cylindrical in the remaining 36 species. It is clear that the cylindrical form is the dominant type among the Indian species and is found in the subgenus *Indigofera*. In the cylindrical type the ends of the seed may be round (er) or truncate (et) or both.

The discoid form incorporates *I. hochstetteri* of the subgenus *Amecarpus*, and *I. zollingeriana* of the subgenus *Indigofera*. The seed is discoid in *I. hochstetteri* because the pods are bilaterally compressed, whereas in *I. zollingeriana* pod is cylindrical, the seeds are arranged like a pile of coins.

Among the treelet/tree species, *I. zollingeriana* has flat discoid seeds, whereas *I. heterantha* and *I. stachyodes* have cylindrical seeds. In the species with gland-dotted pods, the seeds are boat-shaped as in *I. barberi*, *I. nummularifolia* and *I. tirunelvelica*; spherical type in *I. glandulosa*; bilateral type in *I. prostrata*; and cylindrical type in *I. karuppiana*, *I. pedicellata* and *I. trifoliata*.



SEM studies on seed morphology of *Indigofera*

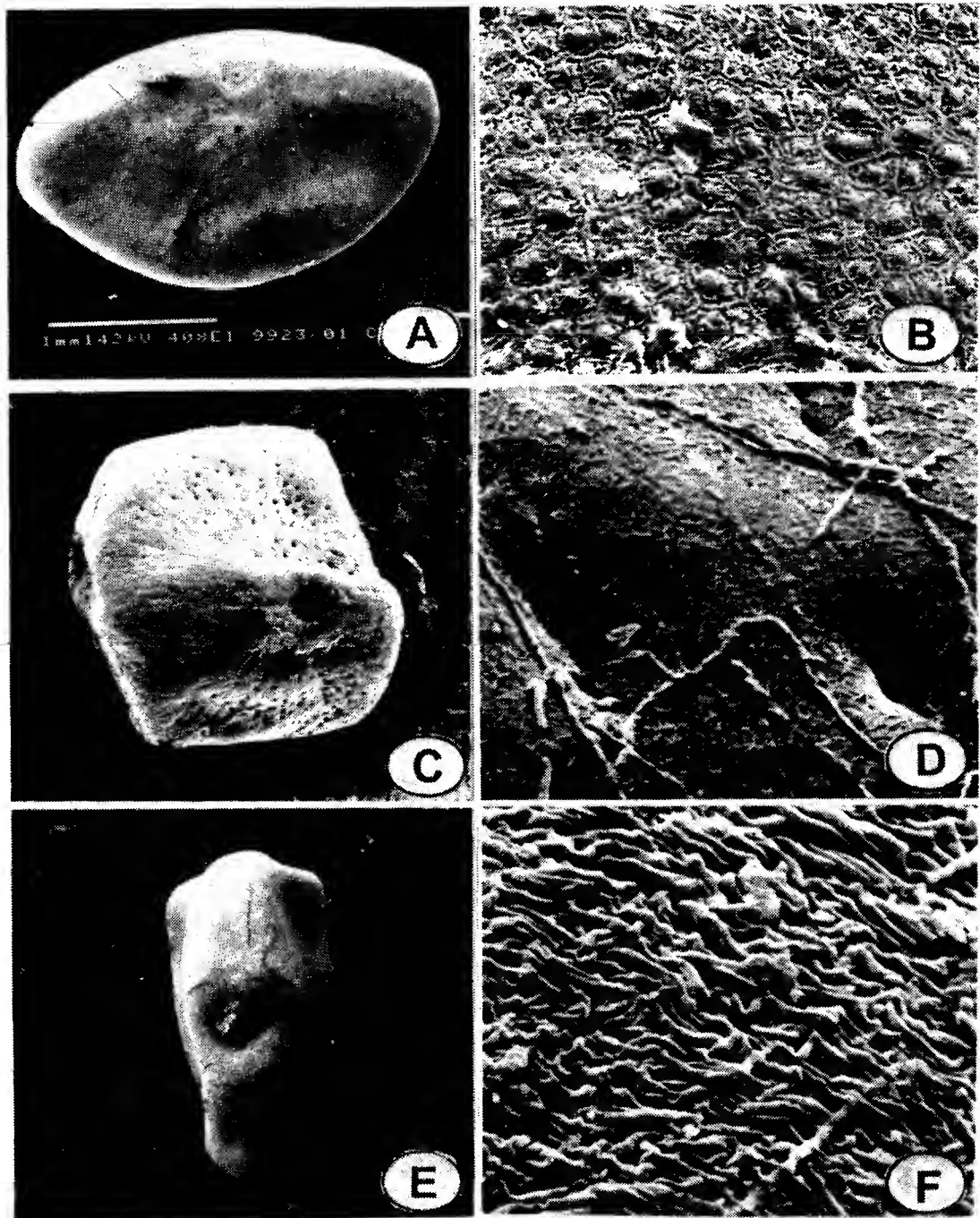


Fig. 1. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A - B. *I. angulosa* (x15, x800); C - D. *I. argentea* (x35, x1000); E - F. *I. arrecta* (x28, x2200).

G.V.S. Murthy and M. Sanjappa

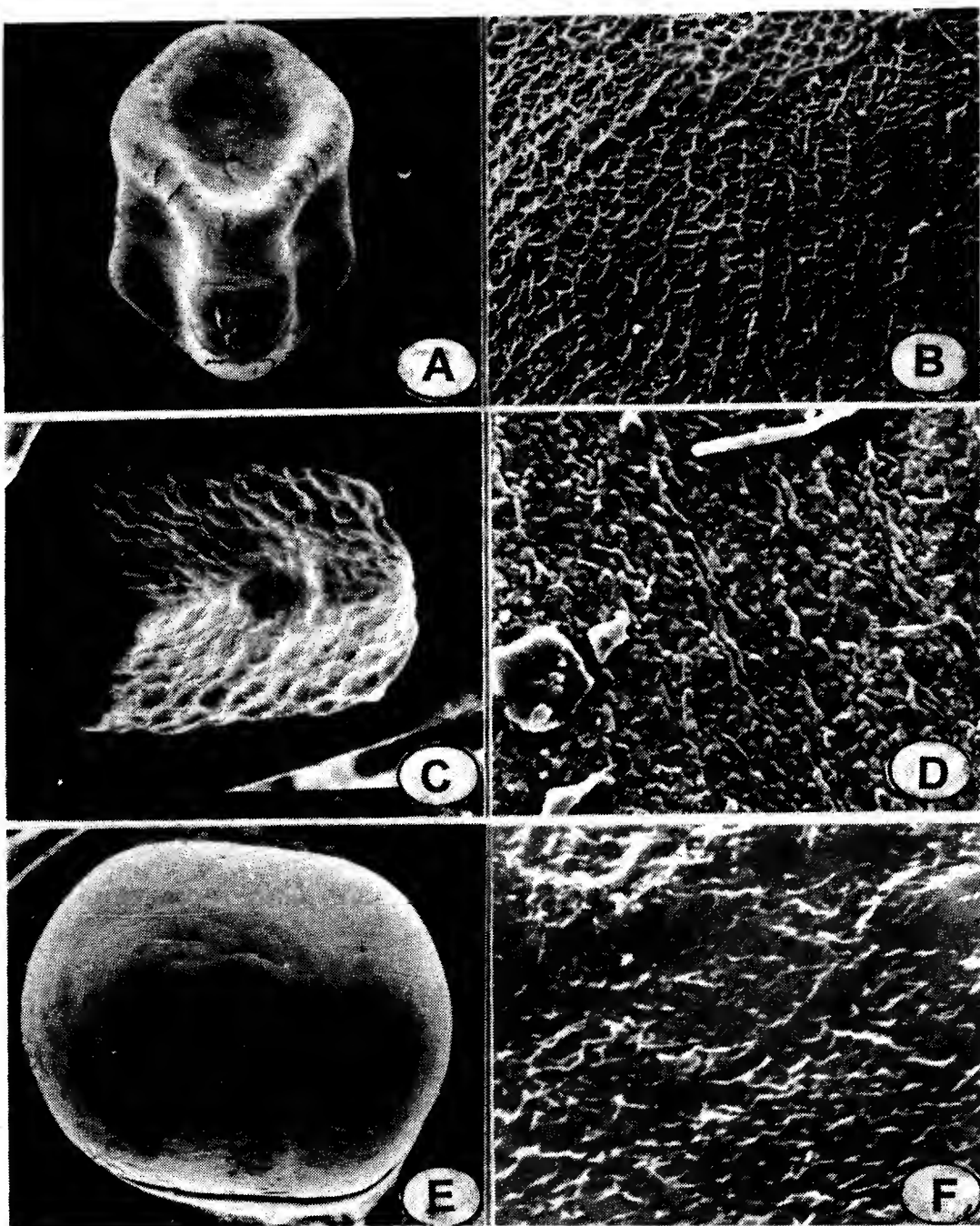


Fig. 2. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. aspalathoides* (x44, x1100); C-D. *I. astragalina* (x20, x2000); E - F. *I. atropurpurea* (x20, x2500).

SEM studies on seed morphology of *Indigofera*

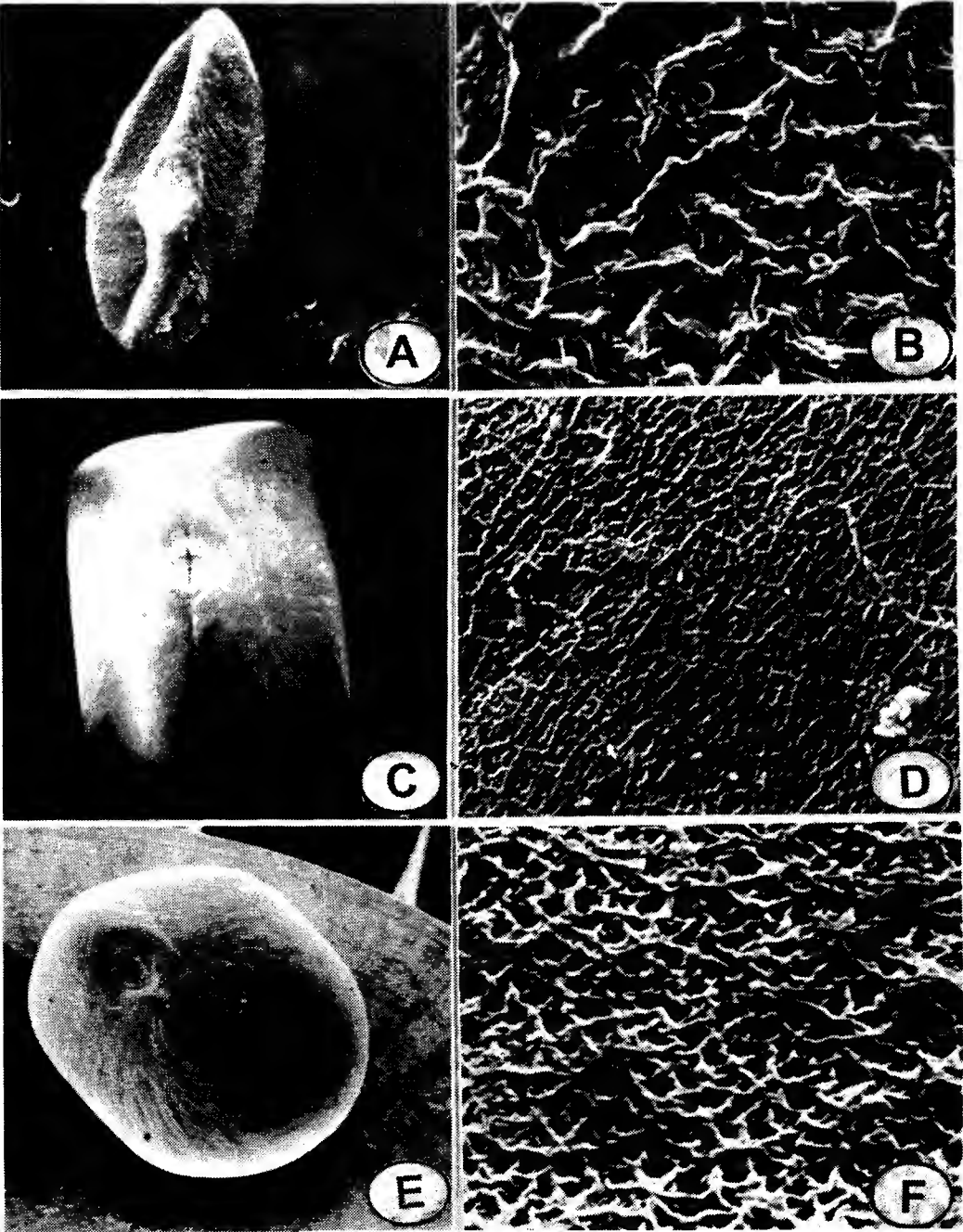


Fig. 3. Scanning electron micrographs of seed and seed surface of *Indigofera*: A-B. *I. barberi* (x33, x530); C-D. *I. bracteata* (x27, x1000); E - F. *I. cassioides* (x18, x2300).

G.V.S. Murthy and M. Sanjappa

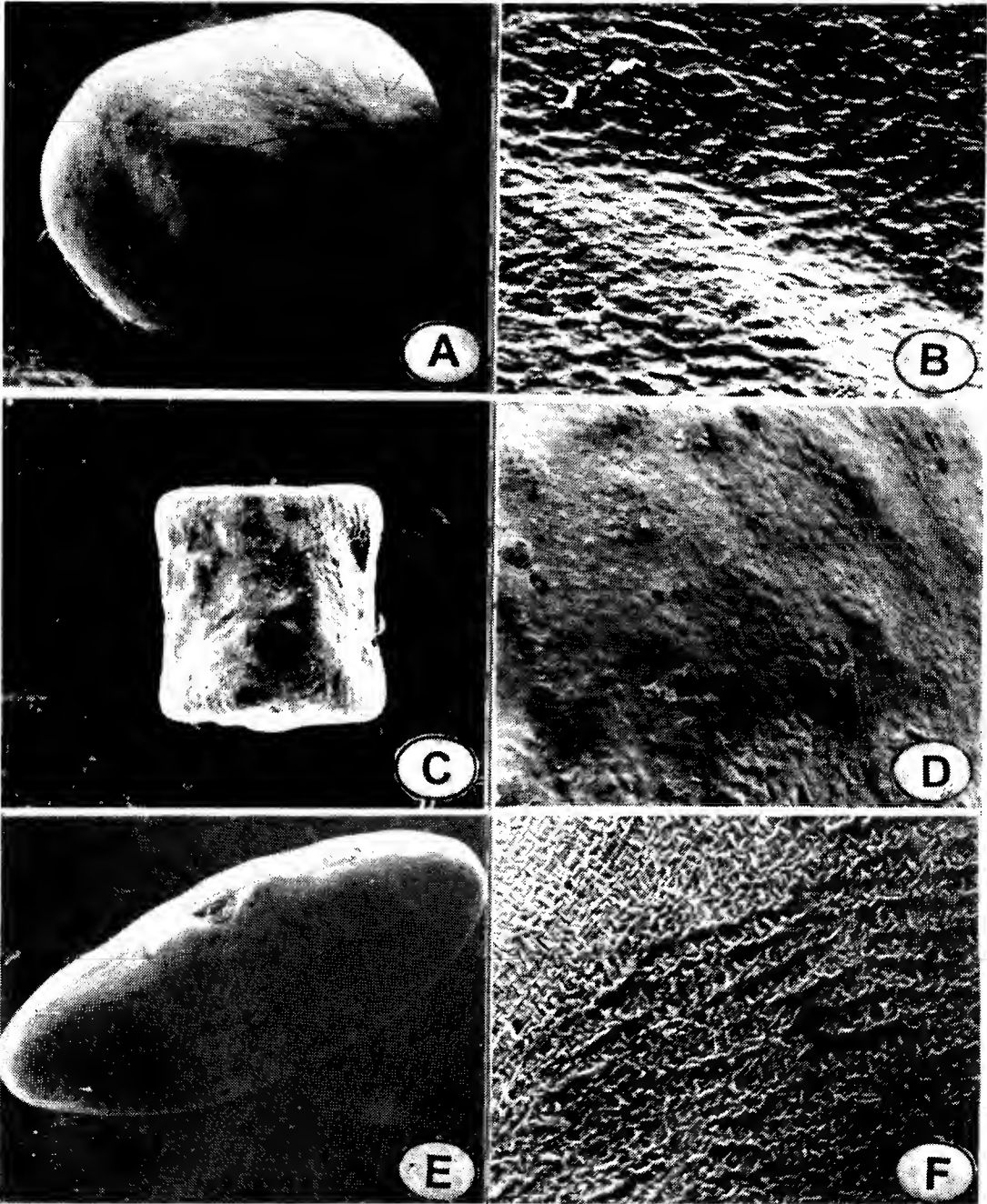


Fig. 4. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. coerulea* (x24, x1100); C-D. *I. colutea* (x35, x1300); E-F. *I. constricta* (x17, x550).

SEM studies on seed morphology of *Indigofera*

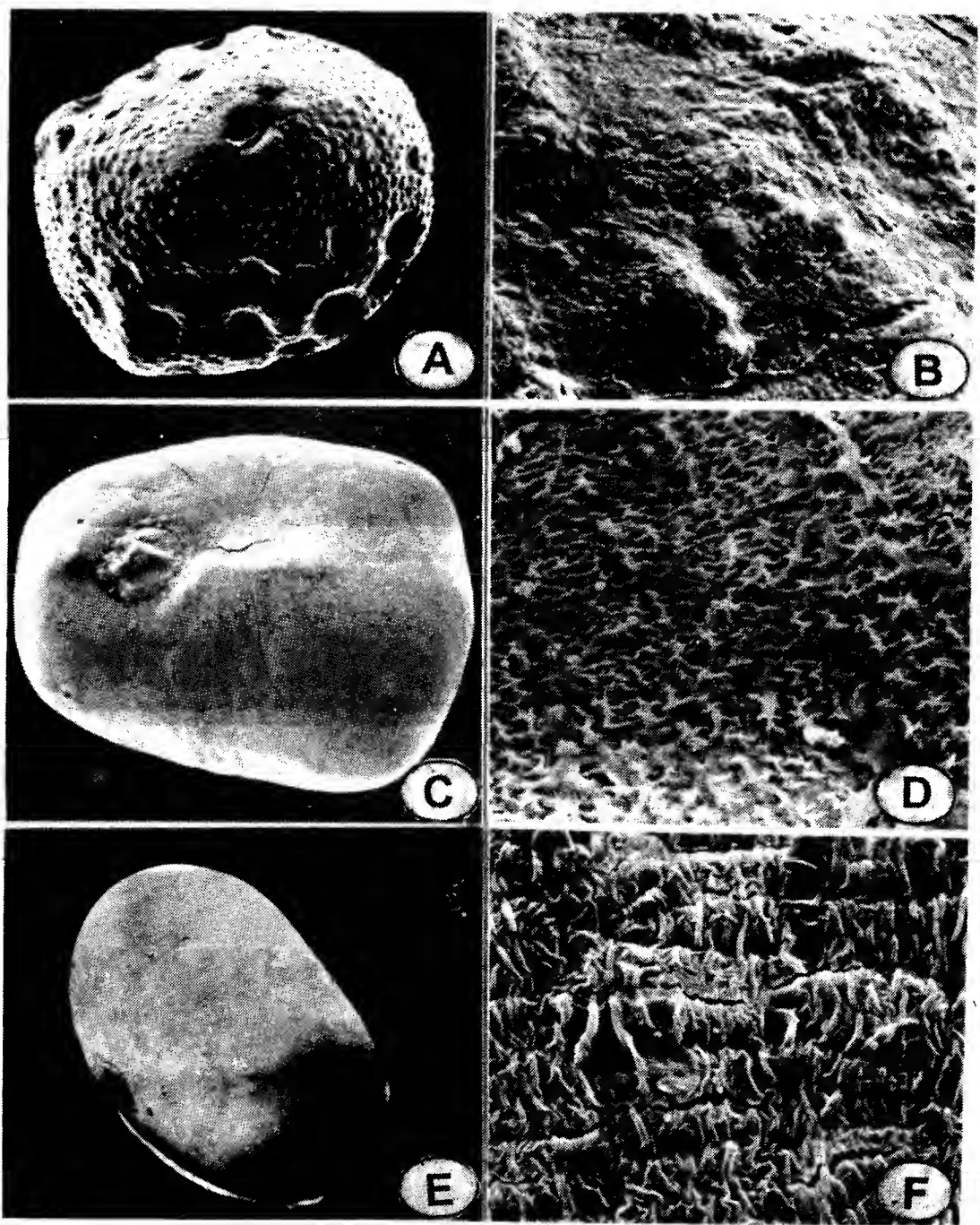


Fig. 5. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. cordifolia* (x42, x1100); C-D. *I. dalzellii* (x35, x1100); E-F. *I. dosua* (x17, x2100).



G.V.S. Murthy and M. Sanjappa

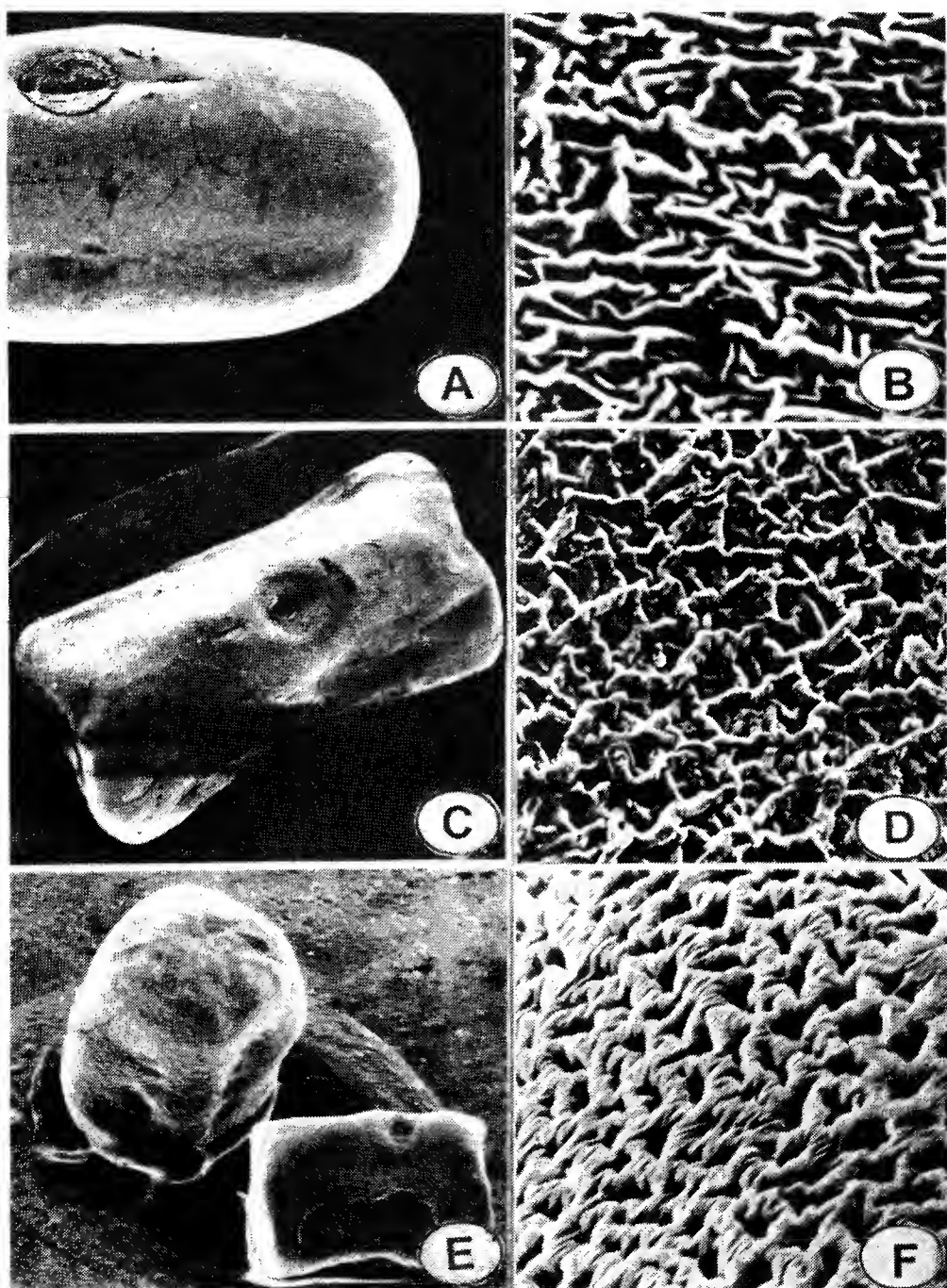


Fig. 6. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A - B. *I. exilis* (x25, x4200); C - D. *I. galeoides* (x21, x2100); E - F. *I. glabra* (x27, x1300).

SEM studies on seed morphology of *Indigofera*

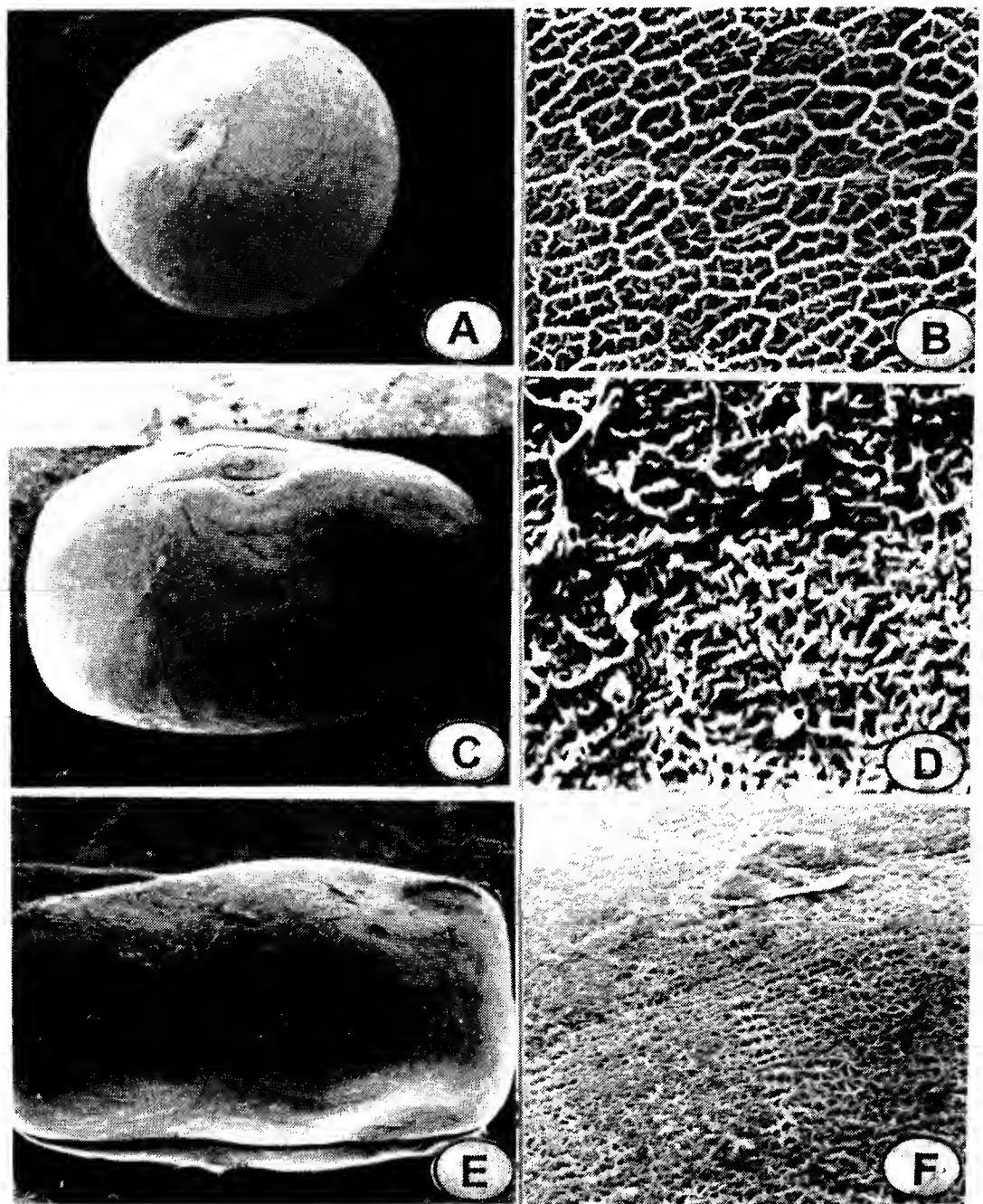


Fig. 7. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. glandulosa* (x25, x1100); C-D. *I. hebepetala* (x17, x1000); E-F. *I. heterantha* (x28, x570).

G.V.S. Murthy and M. Sanjappa

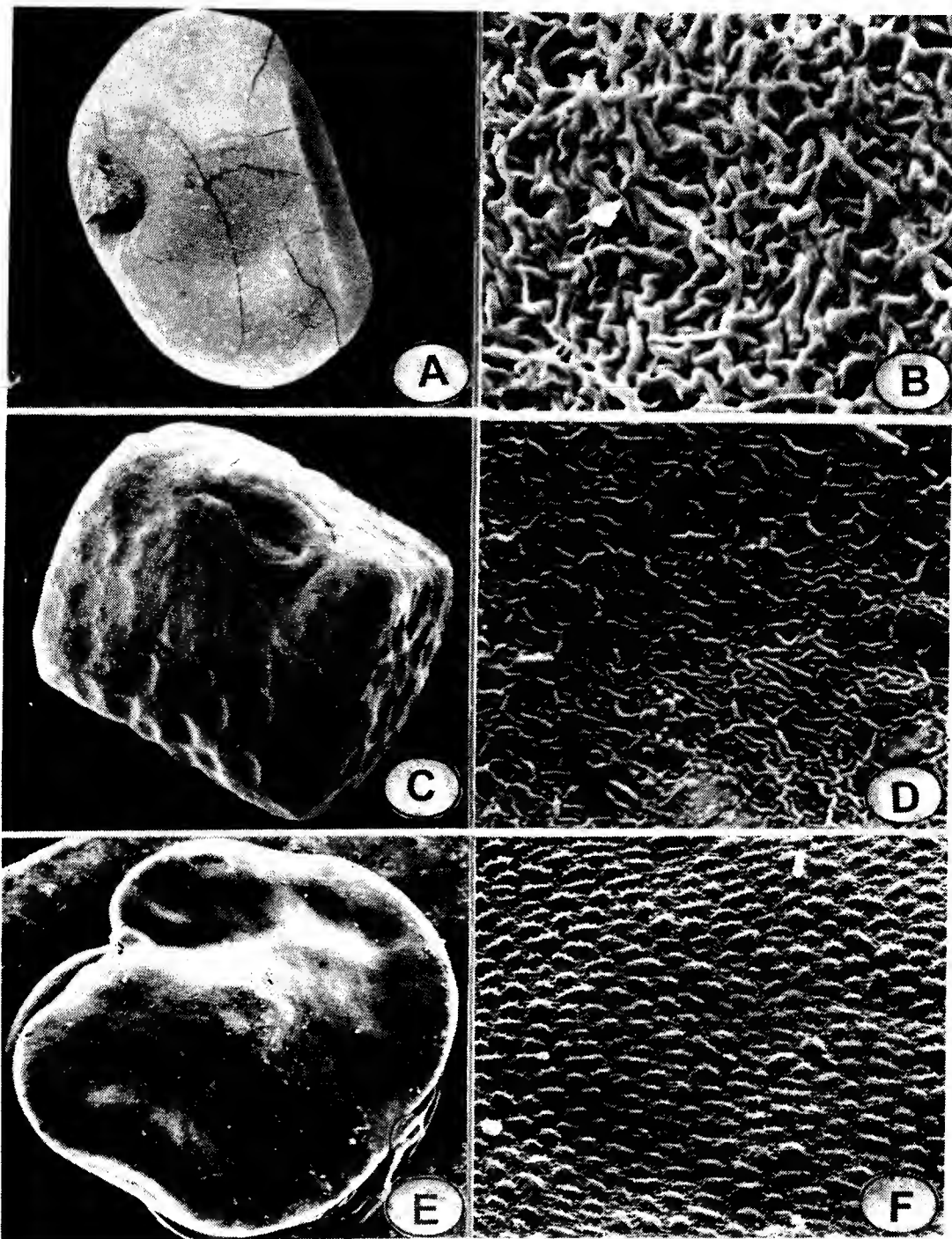


Fig. 8. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. himalayensis* (x29, x2000); C-D. *I. hirsuta* (x33, x4200); E-F. *I. hochstetteri* (x34, x560).



SEM studies on seed morphology of *Indigofera*

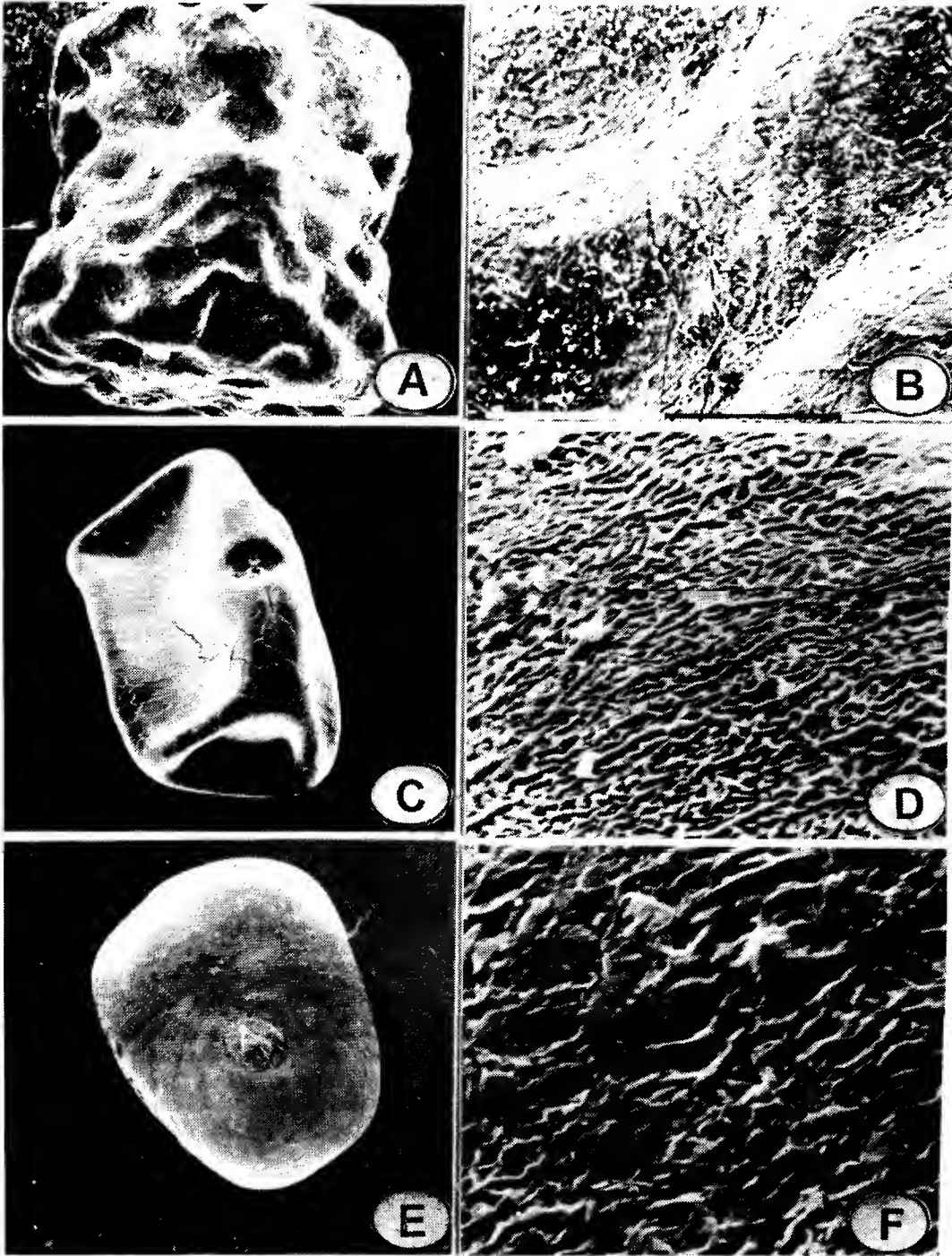


Fig. 9. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. karnatakana* (x35, x250); C - D. *I. karuppiana* (x32, x2200); E - F. *I. lacei* (x25, x4100).

G.V.S. Murthy and M. Sanjappa

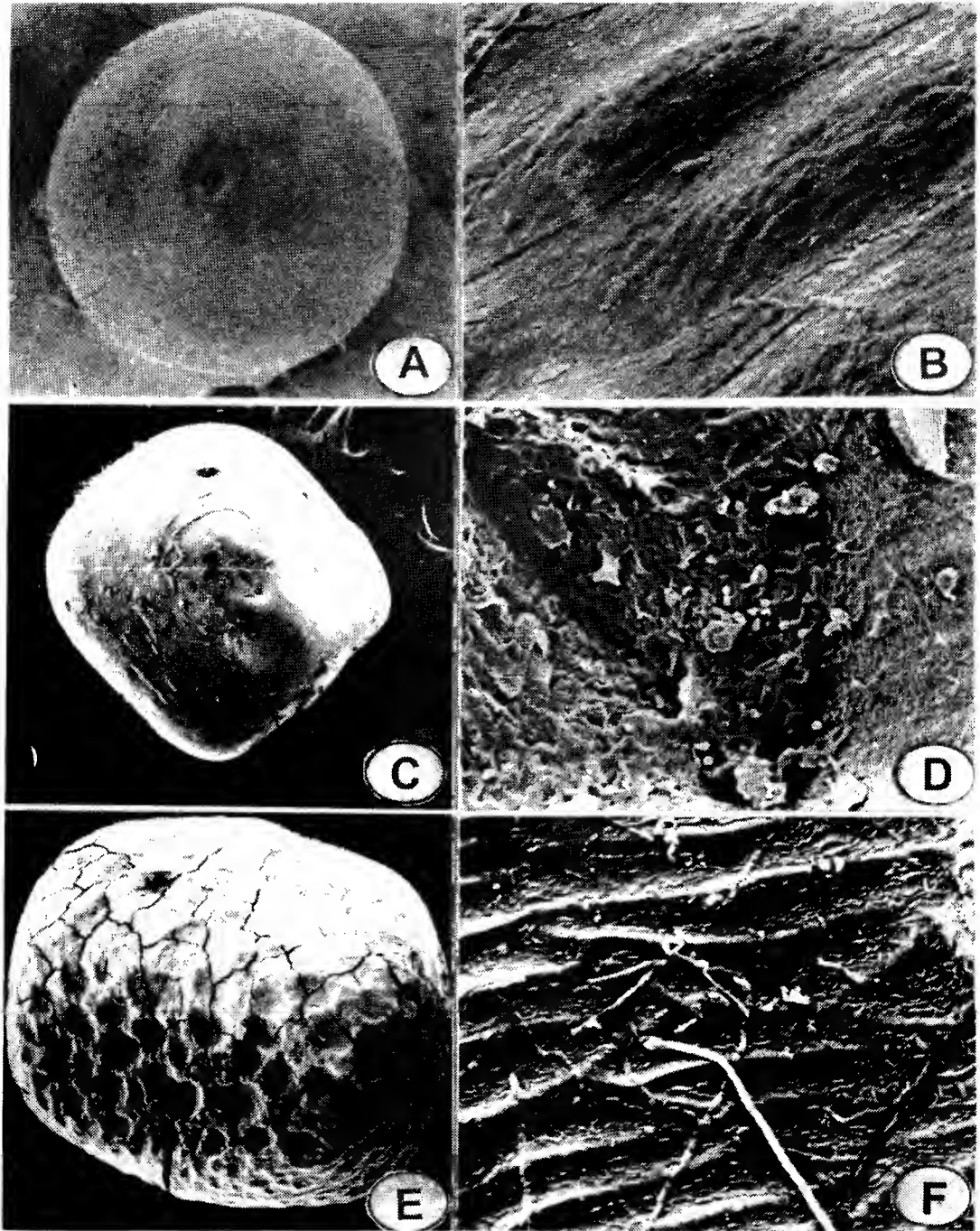


Fig. 10. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. linifolia* (x35, x1100); C-D. *I. linnaei* (x35, x560); E - F. *I. longiracemosa* (x35, x1100).

SEM studies on seed morphology of *Indigofera*

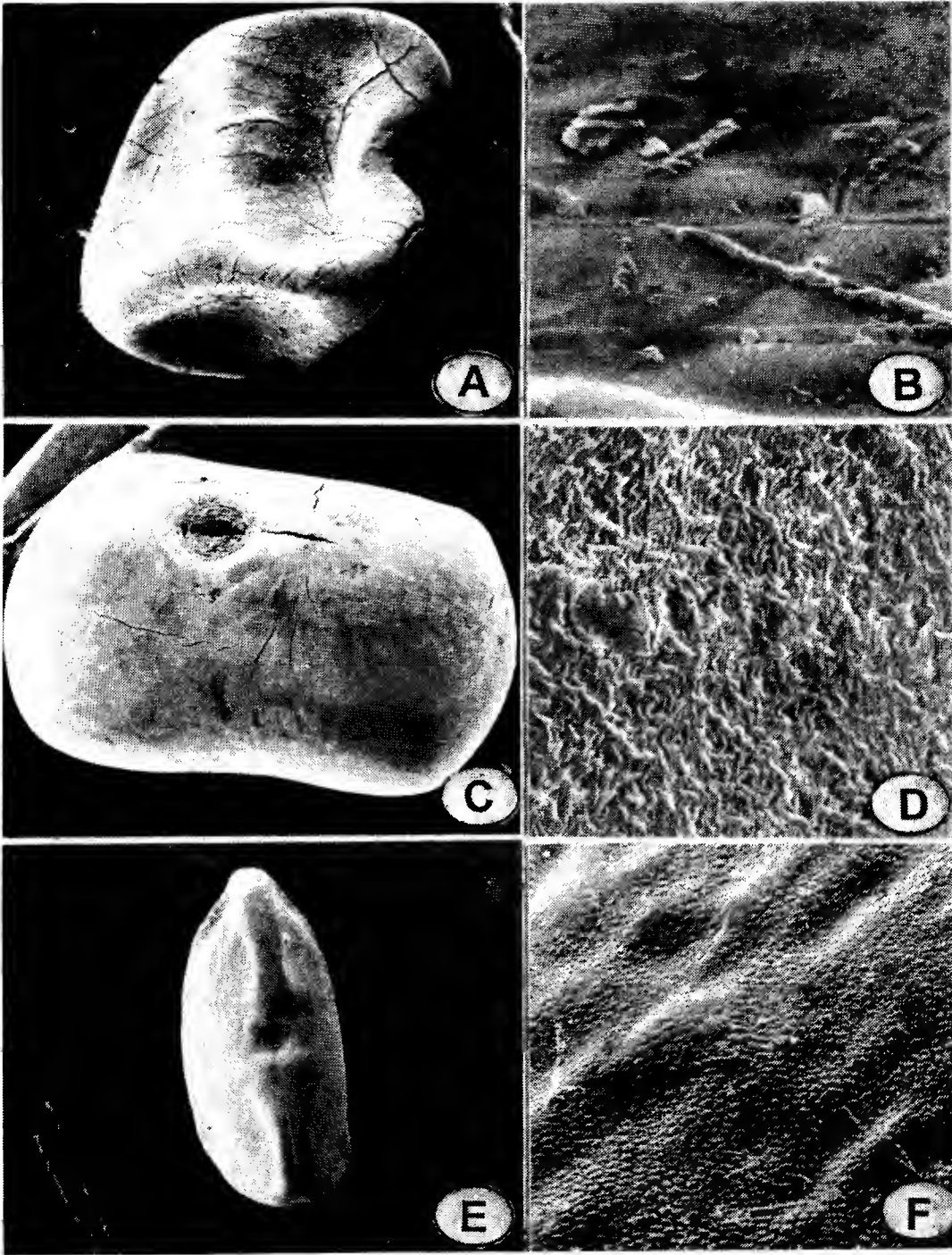


Fig. 11. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. mysorensis* (x32, x2100); C-D. *I. nigrescens* (x27, x2200); E-F. *I. nummulariifolia* (x16, x270).

G.V.S. Murthy and M. Sanjappa

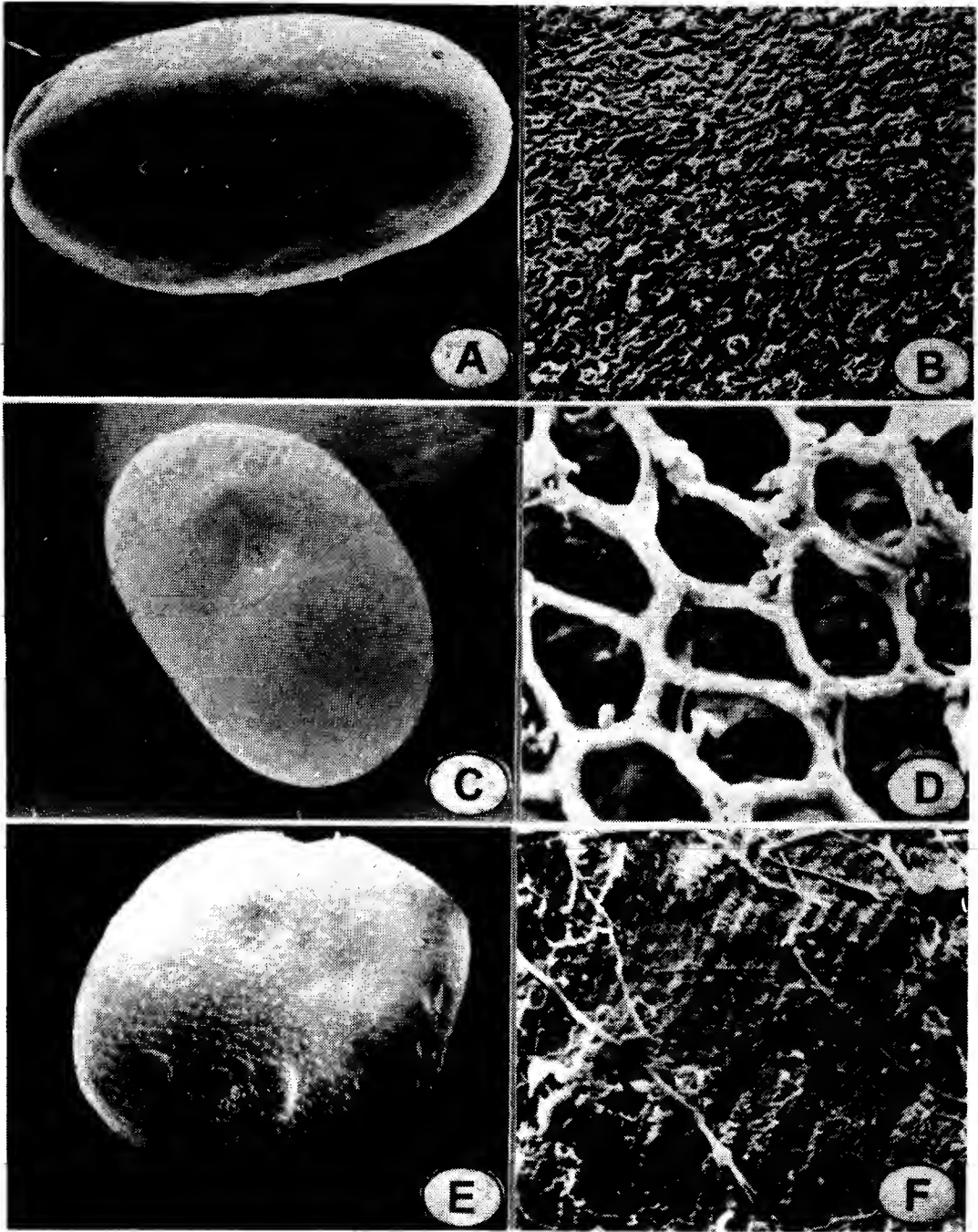


Fig. 12. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. oblongifolia* (x38, x1200); C-D. *I. parviflora* (x28, x4300); E-F. *I. pedicellata* (x37, x670).



SEM studies on seed morphology of *Indigofera*

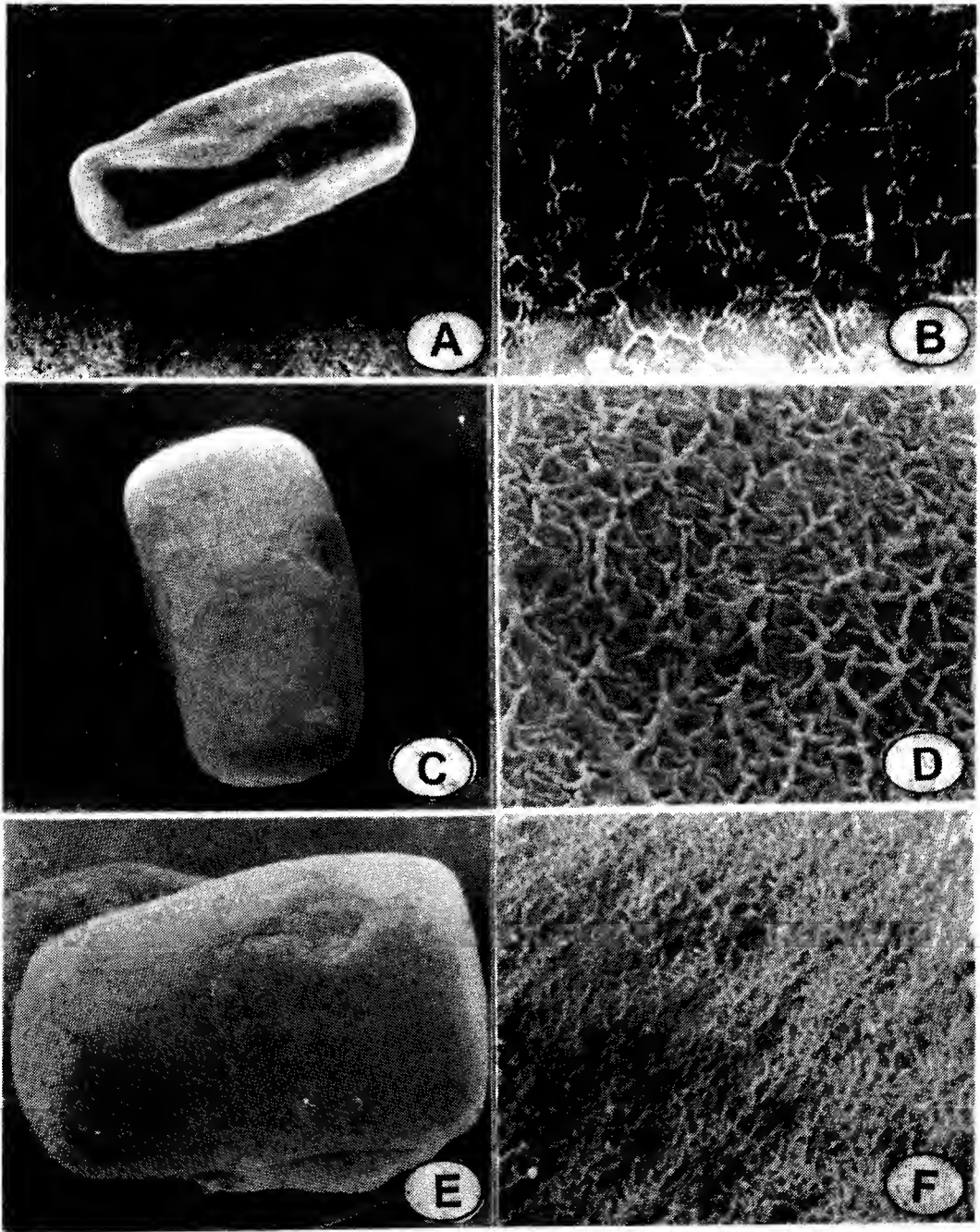


Fig. 13. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. prostrata* (x35, x2200); C - D. *I. pseudoreticulata* (x28, x2100); E - F. *I. sesquipedalis* (x35, x1100).

G.V.S. Murthy and M. Sanjappa

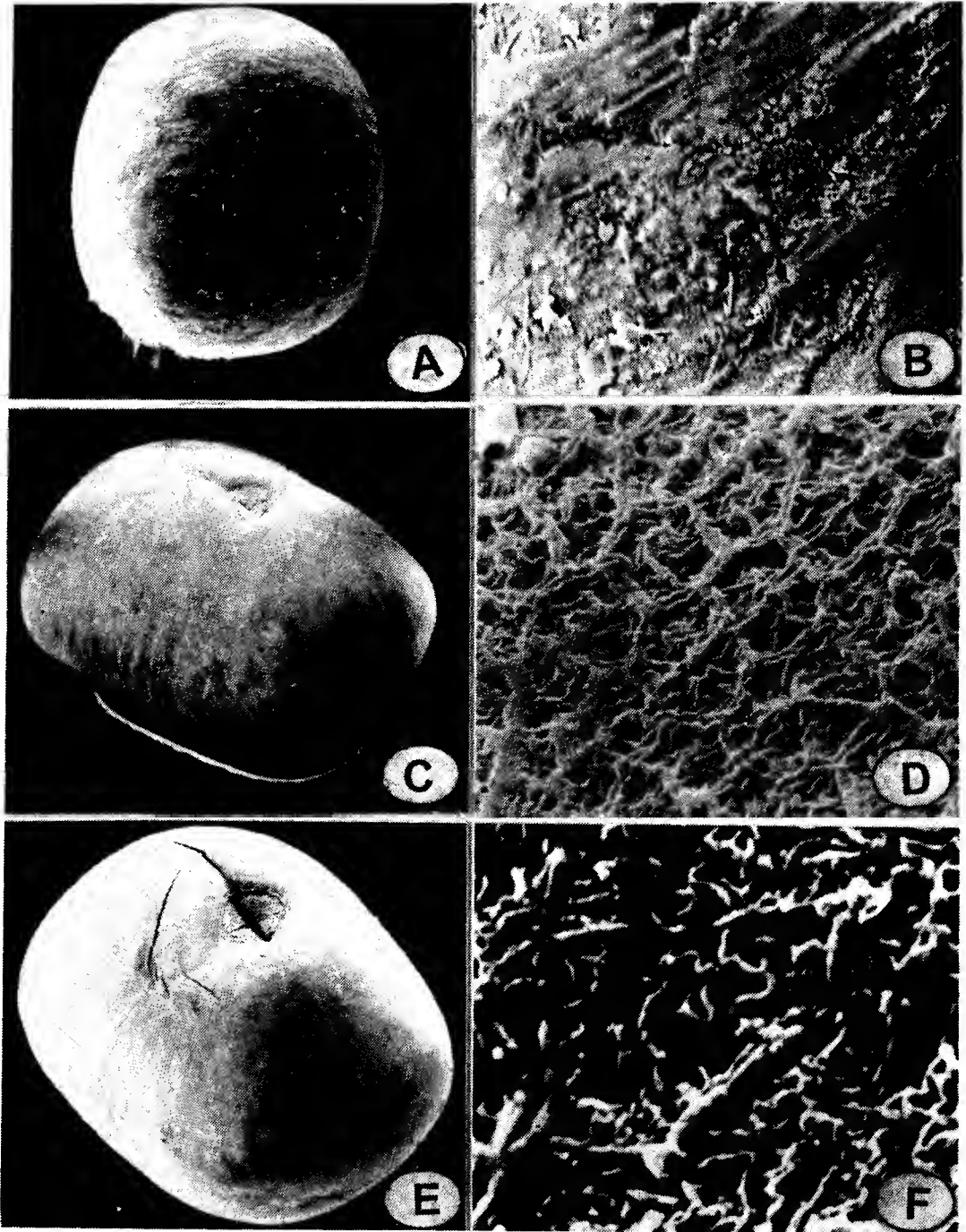


Fig. 14. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. sessiliflora* (x36, x520); C-D *I. spicata* (x26, x2100); E-F. *I. stachyodes* (x29, x4400).

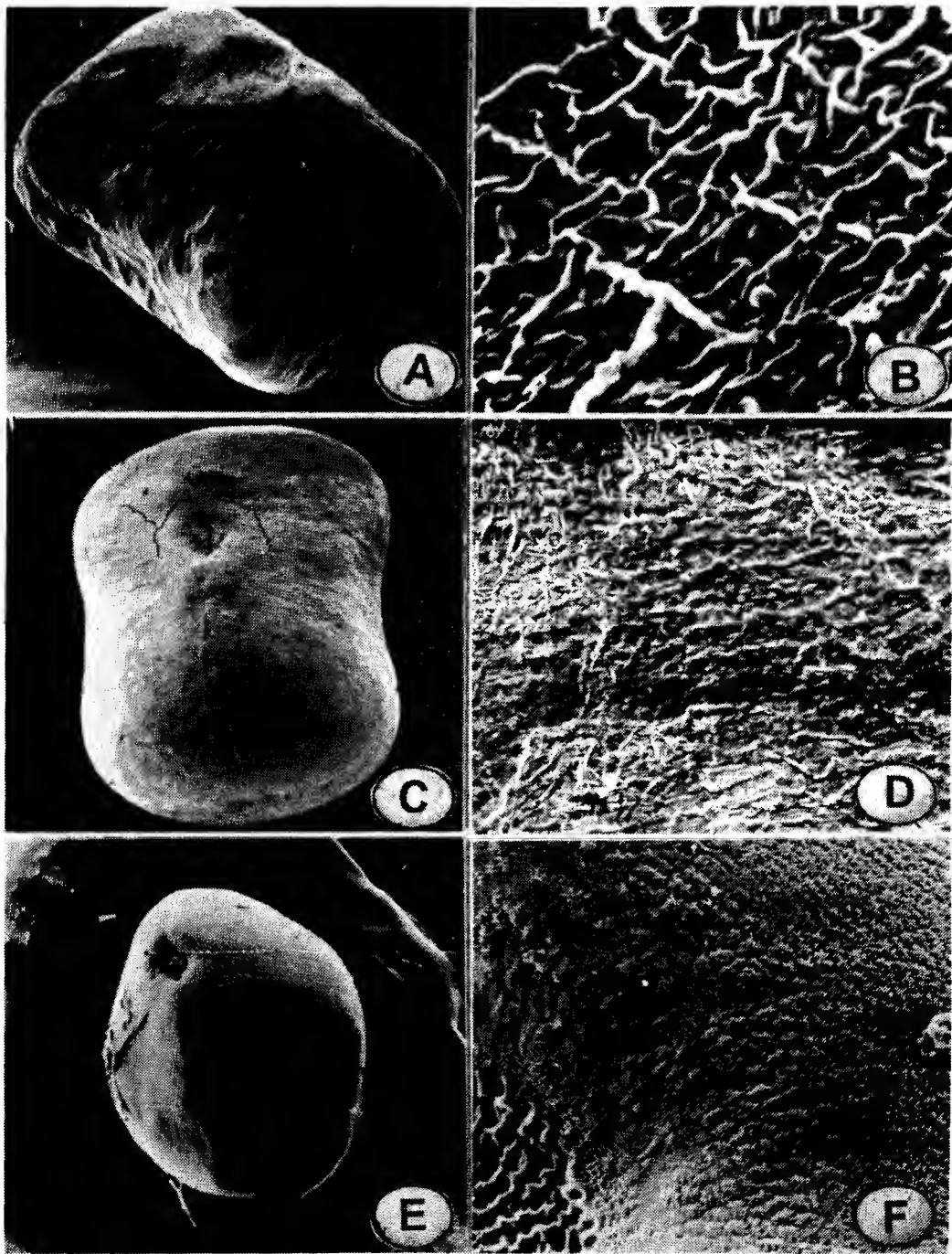
SEM studies on seed morphology of *Indigofera*

Fig. 15. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. suffruticosa* (x31, x4500); C-D. *I. tinctoria* (x25, x1100); E-F. *I. tirunelvelica* (x36, x580).

G.V.S. Murthy and M. Sanjappa

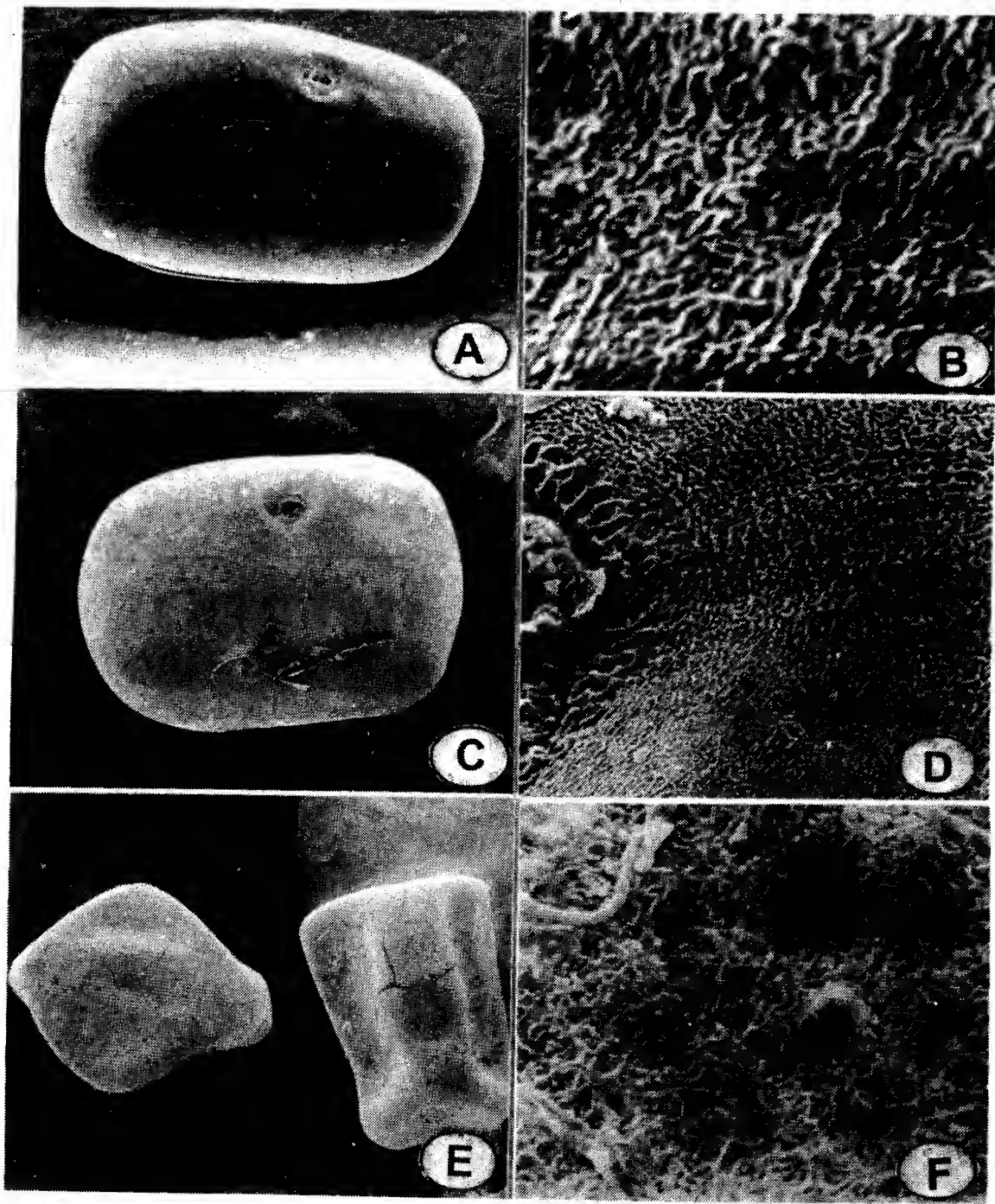


Fig. 16. Scanning electron micrographs of seeds and seed surface of *Indigofera* : A-B. *I. trifoliata* (x36, x4600); C-D. *I. trita* (x35, x560); E-F. *I. uniflora* (x34, x4200).



SEM studies on seed morphology of *Indigofera*

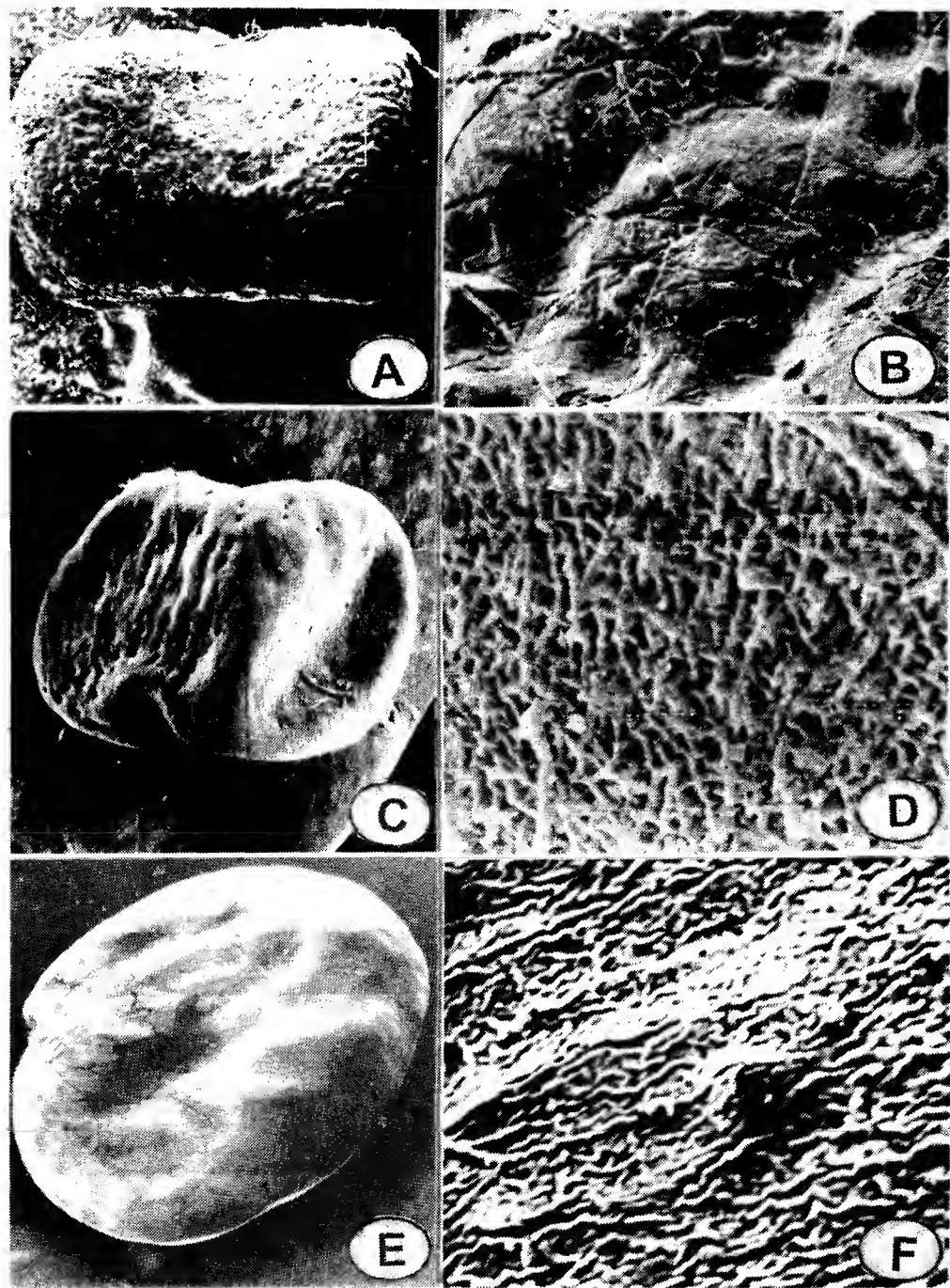


Fig. 17. Scanning electron micrographs of seeds and seed surface of *Indigofera*: A-B. *I. vicioides* (x34, x250); C-D. *I. wightii* (x26, x1700); E-F. *I. zollingeriana* (x28, x4200).

## G.V.S. Murthy and M. Sanjappa

## Seed coat

At lower magnification the seed surface is smooth, pitted, verrucate-pitted or granulate. At higher magnification it is found that surface is either granulate, verrucate, rugulate, reticulate, striato-reticulate, psilate or perforate. The seed surface pattern varied from species to species. The surface of the seed coat is smooth (psilate) in only one species viz., *I. mysorensis* (Fig. 11, B); marked by numerous small perforations in *I. argentea* and *I. wightii* (Fig. 1, C; 17, C); honeycomb like network of ridges in *I. parviflora* (Figs. 12, C - D) which is readily separable from rest of the species and is now placed under a separate genus *Indigastrum* by Schrire (1992); reticulate-papillose in *I. angulosa* (Fig. 1, B); stellate-tuberculate in *I. dalzellii* (Fig. 5, D), *I. heterantha* (Fig. 7, F), *I. nummulariifolia* (Fig. 11, F), and *pseudoreticulata* (Fig. 13, D); matted type in *I. arrecta* (Fig. 1, F) and *I. himalayensis* (Fig. 8, B), while it is wrinkled or folded in *I. dosua* (Fig. 5, F).

Although seed surface in the cylindrical group is quite variable, at low magnification two broad categories such as pitted type and smooth type could be distinguished. The cylindrical pitted type can be further grouped into pitted-verrucate type as seen in *I. astragalina* (Fig. 2, C), *I. colutea* (Fig. 4, C), *I. cordifolia* (Fig. 5, A), *I. glabra* (Fig. 6, E), *I. hirsuta* (Fig. 8, C), *I. longiracemosa* (Fig. 10, E), *I. pedicellata* (Fig. 12, E) and *I. vicioides* (Fig. 17, A); and the second group pitted-smooth type as in *I. karnatakana* (Fig. 9, A) and *I. suffruticosa* (Fig. 15, A). A specialised surface pattern i.e., with concentric rings is seen in *I. uniflora* (Fig. 16, F). The surface is reticulate in most of the species in the genus, however they differ from each other in the size and shape of the reticulum.

Key to the groups/species of *Indigofera* based on seed morphology

- a. Seeds spherical ..... Group 1
  - Seed surface verrucate ..... *I. linifolia*
  - Seed surface psilate ..... *I. sessiliflora*
  - Seed surface reticulate with hexagonal lumina ..... *I. glandulosa*
- b. Seeds boat-shaped ..... Group 2
  - Surface rugose ..... *I. barberi*
  - Surface stellate-verrucate ..... *I. nummulariifolia* and *I. tirunelvelica*
  - Surface striato-reticulate ..... *I. constricta*
  - Surface coarsely reticulate-papillose ..... *I. angulosa*
- c. Seeds discoid ..... Group 3
  - Surface verrucate-reticulate ..... *I. hochstetteri*
  - Surface striato-rugulose ..... *I. zollingeriana*
- d. Seeds bilateral ..... Group 4
  - Seeds with perforations
    - Surface reticulate-faveolate ..... *I. wightii*
    - Surface with faint reticulation ..... *I. argentea*
  - Seeds without perforations
    - Surface reticulate-perforate ..... *I. himalayensis*

SEM studies on seed morphology of *Indigofera*

	Surface psilate .....	<i>I. mysorensis</i>
	Surface coarsely reticulate .....	<i>I. prostrata</i>
e. Seeds cylindrical .....	Group 5	
Seed coat with pits		
Surface pitted and smooth .....	<i>I. karnatakana</i> and <i>I. suffruticosa</i>	
Surface pitted and rough		
Surface granulate .....	<i>I. astragalina</i>	
Surface reticulate .....	<i>I. colutea</i> and <i>I. glabra</i>	
Surface striate .....	<i>I. longiracemosa</i>	
Surface verrucate-psilate .....	<i>I. cordifolia</i> and <i>I. vicioides</i>	
Surface rugose .....	<i>I. hirsuta</i>	
Surface verrucate, verrucae in rows .....	<i>I. pedicellata</i>	
Seed coat without pits		
Surface matted type .....	<i>I. arrecta</i>	
Surface rugulose .....	<i>I. atropurpurea</i> , <i>I. lacei</i> , <i>I. karuppiana</i> and <i>I. nigrescens</i>	
Surface reticulate-granulate .....	<i>I. coerulea</i>	
Surface with stellate verrucae .....	<i>I. dalzellii</i> , <i>I. heterantha</i> and <i>I. pseudoreticulata</i>	
Surface striate .....	<i>I. dosua</i>	
Surface reticulate-papillose .....	<i>I. oblongifolia</i>	
Surface psilate .....	<i>I. tinctoria</i>	
Surface fossulate .....	<i>I. trifoliata</i>	
Surface with connective rings .....	<i>I. uniflora</i>	
Surface reticulate .....	<i>I. aspalathoides</i> , <i>I. bracteata</i> , <i>I. cassioides</i> , <i>I. exilis</i> , <i>I. galegoides</i> , <i>I. hebeptala</i> , <i>I. linnaei</i> , <i>I. parviflora</i> , <i>I. sesquipedalis</i> , <i>I. spicata</i> , <i>I. stachyodes</i> and <i>I. trita</i>	

## Seed morphology and taxonomy

(a) *Species level*: In most of the Indian floras *I. astragalina* was merged with its close ally *I. hirsuta*, until Gillett (1960) and Sanjappa (1995) discussed the differences and retained them as distinct species. Though the seeds of *I. astragalina* and *I. hirsuta* look similar at low magnification, the seed coat is granulate in the former (Fig. 2, D), and striate-rugose in the latter (Fig. 8, D) respectively at high magnification.

De Kort and Thijsse (1984) have merged *I. barberi* Gamble with *I. trifoliata* L. subsp. *trifoliata*. Sanjappa (1995) treated them as distinct species. The boat-shaped and rugose seeds of *I. barberi* (Figs. 13, 14) are distinctly different from that of *I. trifoliata* which has cylindrical seeds with fossulate surface (Figs. 16, A - B).

## G.V.S. Murthy and M. Sanjappa

Ali (1958) and de Kort and Thijssse (1984) have reduced *I. prostrata* as a synonym of *I. trifoliata*, while Gamble (1918), Santapau (1953) and Sanjappa (1995) have treated it as a distinct species. The coarsely reticulate seed coat of *I. prostrata* (Fig. 13, B) is distinct from *I. trifoliata* (Fig. 16, B). Similarly the seeds of *I. dosua* with striations (Fig. 5, F) is different from *I. stachyodes* which has rugulose seeds (Fig. 14, F).

(b) **Subgeneric level:** It is significant that *I. nummulariifolia* of subgenus *Acanthonotus* and *I. hochstetteri* of subgenus *Amecarpus* of Baker (1876) and Gillett (1958) show distinct seed morphology. Each species of the subgenus *Indigofera sensu* Gillett exhibit a characteristic seed morphology (with few exceptions) and thus the typical subgenus in general showed greatest variability, and it includes all seed types: spherical type as in *I. glandulosa*, *I. linifolia* and *I. sessiliflora*; boat-shaped type in *I. angulosa*, *I. barberi*, *I. constricta* and *I. tirunelvelica*; discoid type in *I. zollingeriana*; bilateral type in *I. argentea*, *I. himalayensis*, *I. mysorensis*, *I. prostrata* and *I. wightii*; and cylindrical type in others.

The boat-shaped seed of subgenus *Acanthonotus* (*I. nummulariifolia*, Fig. 11, E-F) is different in shape and ornamentation from the boat-shaped seeds in the subgenus *Indigofera*, represented by *I. angulosa* (Fig. 1, A-B), *I. barberi* (Fig. 3, A-B), *I. constricta* (Fig. 4, E-F) and *I. tirunelvelica* (Fig. 15, E-F).

Seed of *I. linifolia* is spherical with verrucate surface (Fig. 10, A-B) and is distinct among the species studied here. This correlates Baker's (1876) placement of *I. linifolia* under subgenus *Sphaeridiophora*.

The subgenera *Acanthonotus*, *Amecarpus* and *Indigastrum* are represented by one species each in Indian flora seems to differ considerably from subgenus *Indigofera* in seed morphology, and they form a different sequence of specilisation. The seeds with pits are found only in the subgenus *Indigofera*, where the endocarp is often dark spotted, causing embellishments on the seed coat.

The analysis of available data has shown that the study of seed morphology is interesting because of its diversity and suggests parallel evolution in *Indigofera*. It is beyond the scope of this present analysis to critically revise the systematics and phylogeny of the *Indigofera* based on a study of just 51 species of estimated 700 species in the world. The morphological peculiarities are more often restricted to the level of species.

### Acknowledgements

The authors are thankful to the former Director, Botanical Survey of India, Kolkata for facilities and encouragement.

SEM studies on seed morphology of *Indigofera*

## Literature Cited

- Ali, S. I. 1958. Revision of the genus *Indigofera* L. from W. Pakistan, and N.W. Himalayas. *Bot. Notiser* 3 (3): 543-577.
- Ali, S. I. 1977. Papilionaceae. In: E. Nasir and S.I. Ali (Eds.), *Fl. W. Pakistan, No. 100*. University of Karachi.
- Baker, E. G. 1926. *The Leguminosae of Tropical Africa. Gent.* 1: 95-166.
- Baker, J. G. 1871. Papilionaceae. In: D. Oliver (Ed.), *Flora of Tropical Africa*. Reeve, London. 2: 65-103.
- Baker, J. G. 1876. Leguminosae. In: J.D. Hooker (Ed.), *The Flora of British India*. Reeve, London. Vol. 2: 92-102.
- Bentham, G. 1865. Leguminosae. In: G. Bentham & J.D. Hooker, *Genera Plantarum*. Lovell Reeve & Co., London. 1: 494.
- Bhatt, R. P. & M. Sanjappa. 1975. Karyomorphological studies in the genus *Indigofera* Linn. *The Nucleus* 18: 172-177.
- Bir, S. S. & S. Sidhu. 1967. Cytological observations on north Indian members of the family Leguminosae. *The Nucleus* 19: 47-63.
- Brisson, J. D. & R. L. Pterson. 1977. The scanning electron microscope and X-ray microanalysis in the study of seeds: a bibliography covering the period of 1967-1976. *Illinois Inst. Tech. Res. Inst./SEM/1977/II*: 697-712.
- Clarke, C. & J. A. Jernstedt. 1979. Systematic Studies of *Eschscholzia* (Papaveraceae)-II. Seed coat microsculpturing. *Syst. Bot.* 3: 386-402.
- Corner, E. J. H. 1951. The leguminous seed. *Phytomorphology* 1: 117-150.
- Craib, W. G. 1913. The Indigoferas of China. *Not. Roy. Bot. Gard. Edinb.* 36: 47-77.
- Crow, G. E. 1979. The systematic significance of seed morphology in *Sagina* (Caryophyllaceae) under scanning electron microscope. *Brittonia* 31: 52 - 63.
- Darlington, C. D. & A. P. Wylie. 1945. *Chromosome atlas of cultivated plants*. George Allen & Unwin, London. pp.162-163.
- De Candolle, A.P. 1825. *Prodromus Systematis naturalis regni vegetabilis*. Treutte & Wurtz, Paris. 2: 221-233.
- De Kort, I. & Thijssse, G. 1984. A revision of the genus *Indigofera* (Leguminosae - Papilionoideae) in Southeast Asia. *Blumea* 30: 89-151.

## G.V.S. Murthy and M. Sanjappa

- Ferguson, I. K. & R. Strachan. 1982. Pollen morphology and taxonomy of the tribe Indigofereae (Leguminosae : Papilionoideae). *Pollen et Spores* 24: 171-210.
- Frahm-Leliveld, J. A. 1960. Observations on chromosomes in the genus *Indigofera*. *Acta Bot. Neerl.* 9: 286-293.
- Frahm - Leliveld, J. A. 1962. Further observations on chromosomes in the genus *Indigofera*. *Acta Bot. Neerl.* 11: 201-208.
- Frahm - Leliveld, J. A. 1966. Cytotaxonomic notes on the genera *Indigofera* L. and *Cyamopsis* DC. *Genetica* 37: 403-426.
- Gamble, J. S. 1918. In: *Flora of the Presidency of Madras*. Adlard & Sons, London. Vol. 1: 304 - 313.
- Gillett, J.B. 1958. *Indigofera* (*Microcharis*) in Tropical Africa with the related genera *Cymopsis* and *Rhynchotropis*. *Kew Bull. Add. ser.* 1-166.
- Gillett, J. B. 1960. *Indigofera hirsuta* L. & *I. astragalina* DC. *Kew Bull.* 14: 290.
- Harvey, W. H. 1862. Leguminosae. In: W.H. Harvey & O.W. Sonder, *Flora Capensis*. Hodyes Smith & Co., Dublin. 2: 163-203.
- Meyer, E. H. F. 1836. *Commentariorum de Plantis Africane australis*. Leipzig, Konigsberg. pp. 91-108.
- Newell, C. A. & T. Hymowitz. 1978. Seed coat variation in *Glycine* Willd. subgenus *glycine* (Leguminosae) by SEM. *Brittonia* 30: 76-88.
- Rydberg, P. A. 1923. Indigofereae. In: *North American Flora* 24: 137-153.
- Sanjappa, M. 1977. Cytotaxonomical studies in some genera of the tribes Indigofereae and Desmodieae of Fabaceae. Ph. D. thesis, M.S. University, Baroda, India.
- Sanjappa, M. 1995. Leguminosae - Papilionoideae: Tribe Indigofereae. *Fascicles of Flora of India* 21. Botanical Survey of India, Calcutta.
- Sanjappa, M. & R. P. Bhatt. 1976. IOPB chromosome number reports LIII. *Taxon* 25: 494-495.
- Sanjappa, M. & R. P. Bhatt. 1977. IOPB chromosome number reports LVI. *Taxon* 26: 266-267.
- Santapau, H. 1953. Flora of Khandala on the Western Ghats of India. *Rec. Bot. Surv. India* 16: 1-396.
- Schrire, B. D. 1992. New combinations and resurrected names in *Microcharis* and *Indigastrum* (Fabaceae - Papilionoideae). *Bothalia* 22: 165-170.
- Schrire, B. D. 1995. Evolution of the tribe Indigoferae (Leguminosae - Papilionoideae). In: M. Crisp & J. J. Doyle (Eds.), *Advances in Legume Systematics*. Royal Botanic Gardens, Kew. 7: 161-244.

**SEM studies on seed morphology of *Indigofera***

- Singh, A. & R. P. Roy. 1970. Karyological studies in *Trigonella*, *Indigofera* and *Phaseolus*. *The Nucleus* 13: 41-54.
- Taubert, P. 1894. Leguminosae. In: A. Engler & K. Prantl, *Die Natürlichen Pflanzenfamilien*. Wilhelm Engelmann, Leipzig, Berlin. 111(3), 3: 70-338.
- Wight, R. & G.A.W. Arnott. 1834. *Prodromus Florae Peninsulae Indiae Orientalis*. Parbury, Allen & Co. London. pp. 198-205.
- Willdenow, C. L. 1802. *Species Plantarum* (ed. 4). Berlin.